

AMENDMENTS TO THE CLAIMS:

Claims 1-3, 5-7, 9-14, 16, 18-20, 34-36, 40-46, 48-57, 72-75, 91, 108, 109, 113-116, 118-120, 122-129 and 137 are pending in this application. Claims 1, 10, 12, 14, 16-20, 35 and 42 are amended herein. Claims 4, 8 and 17 are cancelled herein without prejudice or disclaimer. Claim 137 is added. This listing of claims will replace all prior versions, and listings of claims, in the application.

LISTING OF CLAIMS:

1. (Currently amended) A substantially purified single-chain ~~single-chain~~ polypeptide, comprising a membrane-type serine protease (MTSP) portion that is a the protease domain of a type-II MTSP ~~membrane-type serine protease (MTSP)~~ or a catalytically active portion of an MTSP protease domain, wherein:

~~the MTSP portion of the protein consists essentially of the protease domain of the MTSP or a catalytically active portion thereof~~

the MTSP portion is the only portion of the single-chain polypeptide from the
MTSP; and

the MTSP portion has serine protease activity.

2. (Previously presented) The substantially purified polypeptide of claim 1, wherein the MTSP is not expressed on endothelial cells.

3. (Original) The substantially purified polypeptide of claim 1, wherein the MTSP is not expressed on normal endothelial cells *in vivo*.

4. (Cancelled).

5. (Original) The substantially purified polypeptide of claim 1 that consists essentially of the protease domain of an MTSP or a catalytically active portion of the protease domain.

6. (Original) The substantially purified polypeptide of claim 1, wherein the expression and/or activity of the MTSP in tumor cells differs from its level of expression and/or activity in non-tumor cells.

7. (Original) The substantially purified polypeptide of claim 1, wherein the MTSP is detectable in a body fluid at a level that differs from its level in body fluids in a subject not having a tumor.

8. (Cancelled).

9. (Original) The substantially purified polypeptide of claim 1, wherein the MTSP exhibits altered substrate specificity in the tumor compared to its specificity in a non-tumor cell in the same tissue.

10. (Currently amended) The substantially purified polypeptide of claim 1, wherein the MTSP portion has an N-terminus that comprises IVNG, ILGG, VGLL or ILGG.

11. (Original) The substantially purified polypeptide of claim 1, wherein the MTSP is selected from among MTSP1, MTSP3, MTSP4 and MTSP6.

12. (Currently amended) The substantially purified polypeptide of claim 1, wherein ~~the protease domain comprises~~ the MTSP portion is a sequence of amino acids selected from the group consisting of the sequences set forth as amino acids 615-855 of SEQ ID No. 2, as amino acids 205-437 of SEQ ID NO. 4, as the amino acids in SEQ ID No. 6, ~~[[or]]~~ and as amino acids 217-443 in SEQ ID No. 12.

13. (Original) The substantially purified polypeptide of claim 1 that has at least about 40%, 60%, 80%, 90% or 95% sequence identity with a protease domain that comprises the sequence of amino acids set forth as amino acids 615-855 of SEQ ID No. 2, as amino acids 205-437 of SEQ ID NO. 4, as the amino acids in SEQ ID No. 6, or as amino acids 217-443 in SEQ ID No. 12.

14. (Currently amended) A polypeptide of claim 1, wherein the MTSP ~~protease domain~~ portion is encoded by a nucleic acid molecule that hybridizes under conditions of high stringency along its full length to a nucleic acid molecule comprising a sequence of nucleotides set forth in SEQ ID No: 1, 3, 5, 7, 9 or 11 or to a molecule that encodes the protein set forth in SEQ ID No: 2, 4, 6, 8, 10 or 12 or ~~at least one~~ the protease domain thereof.

15. (Cancelled).

16. (Currently amended) ~~A mutant of the~~ The polypeptide of claim 1, wherein: up to about ~~[[90%]]~~ 60% of the amino acids of the MTSP portion of the polypeptide are replaced with another amino acid;

the MTSP portion includes an active site triad; and

the resulting polypeptide is a single chain and has catalytic activity at least 10% of the unmutated unmodified polypeptide on a substrate therefore.

17. (Cancelled).

18. (Currently amended) The ~~mutatein~~ polypeptide of claim 16, wherein the resulting polypeptide is a single chain and has catalytic activity at least 50% of the unmutated polypeptide.

19. (Currently amended) ~~A mutatin of the~~ The polypeptide of claim 1, wherein a free Cys in the protease domain is replaced with another amino acid, whereby the resulting polypeptide exhibits proteolytic activity.

20. (Currently amended) ~~A mutatin of the~~ The polypeptide of claim 1, wherein a free Cys in the protease domain is replaced with a serine.

Claims 21- 33 (Cancelled).

34. (Previously presented) The polypeptide of claim 1, wherein the MTSP is selected from among corin, MTSP1, enteropeptidase, human airway trypsin-like protease (HAT), TMPRSS2, and TMPRSS4.

35. (Currently amended) A conjugate, comprising:

a) a ~~protein~~ polypeptide of claim 1, and

b) a targeting agent linked to the protein directly or via a linker, wherein the conjugate has serine protease activity.

36. (Original) The conjugate of claim 35, wherein the targeting agent permits

i) affinity isolation or purification of the conjugate;

ii) attachment of the conjugate to a surface;

iii) detection of the conjugate; or

iv) targeted delivery to a selected tissue or cell.

Claims 37 – 39 (Cancelled)

40. (Previously presented) A solid support comprising two or more polypeptides of claim 1 linked thereto either directly or via a linker.

41. (Previously presented) The support of claim 40, wherein the polypeptides comprise an array.

42. (Currently amended) The support of claim ~~40~~ 41, wherein the array comprises polypeptides ~~comprise a plurality of~~ having different MTSP protease domains.

43. (Withdrawn) A method for identifying compounds that modulate the protease activity of an MTSP, comprising:

contacting a polypeptide of claim 1 with a substrate proteolytically cleaved by the MTSP, and, either simultaneously, before or after, adding a test compound or plurality thereof; measuring the amount of substrate cleaved in the presence of the test compound; and

selecting compounds that change the amount cleaved compared to a control, whereby compounds that modulate the activity of the MTSP are identified.

44. (Withdrawn) The method of claim 43, wherein the test compounds are small molecules, peptides, peptidomimetics, natural products, antibodies or fragments thereof.

45. (Withdrawn) The method of claim 43, wherein a plurality of the test compounds are screened simultaneously.

46. (Withdrawn) The method of claim 43, wherein the change in the amount cleaved is assessed by comparing the amount cleaved in the presence of the test compound with the amount in the absence of the test compound.

47. (Cancelled)

48. (Withdrawn) The method of claim 43, wherein a plurality of the polypeptides are linked to a solid support, either directly or via a linker.

49. (Withdrawn) The method of claim 43, wherein the polypeptides comprise an array.

50. (Withdrawn) The method of claim 43, wherein the polypeptides comprise a plurality of different MTSP proteases.

51. (Withdrawn) A method of identifying a compound that specifically binds to a single chain protease domain of an MTSP, comprising:

contacting a polypeptide of claim 1 with a test compound or plurality thereof under conditions conducive to binding thereof; and

identifying compounds that specifically bind to the MTSP single chain protease domain or compounds that inhibit binding of a compound known to bind to the MTSP single chain protease domain, wherein the known compound is contacted with the polypeptide before, simultaneously with or after the test compound.

52. (Withdrawn) The method of claims 51, wherein the polypeptide is linked either directly or indirectly via a linker to a solid support.

53. (Withdrawn) The method of claim 51, wherein the test compounds are small molecules, peptides, peptidomimetics, natural products, antibodies or fragments thereof.

54. (Withdrawn) The method of claim 51, wherein a plurality of the test substances are screened for simultaneously.

55. (Withdrawn) The method of claim 52, wherein a plurality of the polypeptides are linked to a solid support.

56. (Previously presented) A substantially purified membrane-type serine protease 3 (MTSP3).

57. (Previously presented) The MTSP3 of claim 56 that is selected from the group consisting of:

- a polypeptide encoded by the sequence of nucleotides set forth in SEQ ID No. 3;
- a polypeptide encoded by a sequence of nucleotides that hybridizes under conditions of high stringency to the sequence of nucleotides set forth in SEQ ID No. 3;
- a polypeptide that comprises the sequence of amino acids set forth as amino acids 205-437 of SEQ ID No. 4;
- a polypeptide that comprises a sequence of amino acids having at least about 90% sequence identity with the sequence of amino acids set forth in SEQ ID No. 4; and
- a polypeptide encoded by a splice variant of the sequence of nucleotides set forth in SEQ ID No. 3.

Claims 58 – 71 (Cancelled).

72. (Previously presented) A substantially purified membrane-type serine protease 4 (MTSP4).

73. (Previously presented) The substantially purified MTSP4 of claim 72 that is an MTSP4-L or an MTSP4-S.

74. (Previously presented) The MTSP4 of claim 72 that is selected from the group consisting of:

- a polypeptide encoded by the sequence of nucleotides set forth in SEQ ID No. 7 or 9;
- a polypeptide encoded by a sequence of nucleotides that hybridizes under conditions of high stringency to the sequence of nucleotides set forth in SEQ ID No. 7 or 9;
- a polypeptide that comprises the sequence of amino acids set forth in SEQ ID No. 6, 8 or 10; and
- a polypeptide encoded by a splice variant of the sequence of nucleotides set forth in SEQ ID No. 7 or 9.

75. (Previously presented) The MTSP4 of claim 74 that is an MTSP4-L or an MTSP4-S.
Claims 76 – 90 (Cancelled).

91. (Previously presented) A substantially purified membrane-type serine protease 6 (MTSP6) selected from the group consisting of:

- a polypeptide encoded by the sequence of nucleotides set forth in SEQ ID No. 11;

a polypeptide encoded by a sequence of nucleotides that hybridizes along the full length thereof under conditions of high stringency to the sequence of nucleotides set forth in SEQ ID No. 11;

a polypeptide that comprises the sequence of amino acids set forth as amino acids 217-443 of SEQ ID No. 12; and

a polypeptide encoded by a splice variant of the sequence of nucleotides set forth in SEQ ID No. 11.

Claims 92 – 107 (Cancelled).

108. (Previously presented) A conjugate, comprising:

- a) an MTSP3 or an MTSP4 or the MTSP6 of claim 91; and
- b) a targeting agent linked to the protein directly or via a linker.

109. (Previously presented) The conjugate of claim 108, wherein the targeting agent permits

- i) affinity isolation or purification of the conjugate;
- ii) attachment of the conjugate to a surface;
- iii) detection of the conjugate; or
- iv) targeted delivery to a selected tissue or cell.

Claims 110 – 112 (Cancelled).

113. (Previously presented) A solid support comprising two or more MTSP3 polypeptides and/or MTSP4 polypeptides and/or the MTSP6 polypeptides of claim 90 linked thereto either directly or via a linker

114. (Previously presented) The support of claim 113, wherein the polypeptides comprise an array.

115. (Withdrawn) A method for identifying compounds that modulate the protease activity of an MTSP selected from an MTSP3 or an MTSP4 or the MTSP6 of claim 91, comprising:

contacting the MTSP with a substrate proteolytically cleaved by the MTSP, and, either simultaneously, before or after, adding a test compound or plurality thereof;

measuring the amount of substrate cleaved in the presence of the test compound; and
selecting compounds that change the amount cleaved compared to a control, whereby compounds that modulate the activity of the MTSP are identified.

116. (Withdrawn) The method of claim 115, wherein the test compounds are small molecules, peptides, peptidomimetics, natural products, antibodies or fragments thereof.

117. (Canceled).

118. (Withdrawn) The method of claim 115, wherein the change in the amount cleaved is assessed by comparing the amount cleaved in the presence of the test compound with the amount in the absence of the test compound.

119. (Withdrawn) The method of claim 115, wherein a plurality of the test substances are screened for simultaneously.

120. (Withdrawn) The method of claim 119, wherein a plurality of the polypeptides are linked to a solid support.

121. (Cancelled).

122. (Withdrawn) A method of identifying a compound that specifically binds to an MTSP selected from an MTSP3, an MTSP4 and the MTSP6 of claim 91, comprising:
contacting the MTSP polypeptide with a test compound or plurality thereof under conditions conducive to binding thereof; and
identifying compounds that specifically bind to the MTSP.

123. (Withdrawn) The method of claim 122, wherein the polypeptide is linked either directly or indirectly via a linker to a solid support.

124. (Withdrawn) The method of claim 122, wherein the test compounds are small molecules, peptides, peptidomimetics, natural products, antibodies or fragments thereof.

125. (Withdrawn) The method of claim 122, wherein a plurality of the test substances are screened for simultaneously.

126. (Withdrawn) The method of claim 125, wherein a plurality of the polypeptides are linked to a solid support.

127. (Previously presented) An MTSP6 polypeptide, comprising amino acids set forth as amino acids 46-55 in SEQ ID No. 12 and/or amino acids 368-394 of SEQ ID No. 12, and that is encoded by a sequence of nucleic acids that hybridizes under moderate stringency to nucleic acid encoding the polypeptide set forth in SEQ ID No. 12.

128. (Previously presented) The polypeptide of claim 127 that comprises the amino acids set forth as amino acids 46-55 in SEQ ID No. 12 and/or amino acids 368-394 of SEQ ID No. 12, and that is encoded by a sequence of nucleic acids that hybridizes under high stringency along its full length or full length of the protease domain to nucleic acid encoding the polypeptide set forth in SEQ ID No. 12.

129. (Previously presented) The polypeptide of claim 127, comprising the sequence of amino acids set forth in SEQ ID No. 12.

Claims 130 – 136 (Cancelled).

137. (New) The polypeptide of claim 16, wherein the substrate is selected from the group consisting of pyroGlu-Pro-Arg-pNA·HCl (S 2366), CH₃SO₂-D-HHT-Gly-Arg-pNA·AcOH (spectrozyme t-PA), CH₃SO₂-D-HHT-Gly-Arg-pNA (Pefachrome t-PA), H-D-Phe-Pip-Arg-pNA·2HCl (S 2238), H-D-Val-Leu-Arg-pNA·2HCl (S 2266), H-D-CHT-Gly-Arg-pNA·2AcOH (Spectrozyme fXIIa), H-D-HHT-Ala-Arg-pNA·2AcOH (Spectrozyme THE), pyroGlu-Gly-Arg-pNA·HCl (S2444), N- α -Z-D-Arg-Gly-Arg-pNA·2HCl (S2765), H-D-Ile-Pro-Arg-pNA·2HCl (S 2288), H-D-Pro-Phe-Arg-pNA·2HCl (S 2302), benzoyl-L-isoleucine-L-glutamic acid -[gamma-methyl ester] -L-arginine-p-nitroanalide (S 2222), methoxy-succinyl-L-arginine-L-prolyl-L-tyrosyl-p-nitroanilide (S 2586) and H-D-Val-Leu-Lys-pNA·2HCl (S 2251).